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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/614,505	07/11/00	ROTHBERG J	15966-539-CI

HM22/0416

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EXAMINER	
TAYLOR, I	
ART UNIT	**PAPER NUMBER**
1655	8

DATE MAILED: 04/16/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/614,505	Applicant(s) ROTHBERG ET AL.	
	Examiner Janell Taylor Cleveland	Art Unit 1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 March 2001* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-55* is/are pending in the application.

4a) Of the above claim(s) *1-26* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *27-55* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15) ☒ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *3, 6* .
18) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other: .

DETAILED ACTION

Election/Restrictions

1. Applicant's election without traverse of Group IV, claims 27-55, in Paper No. 7 is acknowledged.

Claim Objections

2. Claim 44 is objected to because of the following informalities: the word "endonuclease" is misspelled. Appropriate correction is required.

Claim Rejections - 35 USC § 112

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 38-42 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The claims are drawn to "the first subset of nucleic acids differ by..." However, it is not specified what they differ from, whether this means they differ from the second subset, or each other, or if they differ in length, base, etc. Appropriate correction is required.

5. Claim 50 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The claim is drawn to "two or more members of the library are combined prior to separating the second subset..." First of all, it is not clear what the members of the library are. Are these members of the first subset, second subset, or a

combination of the two? Secondly, how are they combined? Does this mean the nucleic acids are mixed together, or hybridized together? Clarification is required.

6. Claims 27-55 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The claims are drawn to "a first subset of nucleic acid molecules further comprising at least a second subset of nucleic acid molecules..." However, it is not clear what is meant by this phrase. It is not clear if the second subset is contained within the first subset, i.e. there is a region of the nucleic acid of subset one which is considered to be a part of subset two, or if they are distinct, noncontiguous nucleic acids. Appropriate clarification/correction is required. Furthermore, the claims are drawn to separating the second subset of nucleic acid molecules from "at least some of the other members of the library", however, it would appear from the claim language that only the first subset is in the library, since the other nucleic acids were separated before the library was formed. Therefore, it is not clear how there can be distinguishable characteristics between members of the library, since all of the members originated from the same source of nucleic acid. Clarification is required.

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. Claims 27-42 and 47-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kamb et al. (USPN 6,060,240) in view of Okayama et al (Molecular and Cellular Biology, Vol. 2, No. 2, pages 161-170).

Claim 27 is drawn to a method of identifying a nucleic acid sequence, the method comprising: a) providing a population of nucleic acid molecules comprising at least a first subset of nucleic acid molecules, the first subset of nucleic acid molecules further comprising at least a second subset of nucleic acid molecules; b) separating the first subset of nucleic acid molecules from other nucleic acid molecules in the population of nucleic acid molecules; c) isolating the first subset of nucleic acid molecules; d) constructing a library with the isolated first subset of nucleic acid molecules, wherein one or more members of the library comprises the second subset of nucleic acid molecules and wherein one or more members of the library is distinguishable from at least one of the other members of the library; e) recovering nucleic acids from one or more members of the library; f) separating the second subset of nucleic acid molecules from at least some of the other members in the library; g) isolating at least one nucleic acid molecule from the second subset of nucleic acid molecules; and h) sequencing the nucleic acid molecule, thereby identifying a nucleic acid sequence. Claims 28 and 31 are drawn to the use of primers. Claim 29 is drawn to the nucleic acid molecules being from cDNA. Claim 30 is drawn to the cDNA molecules comprising a library selected from the group consisting of the 5' end, the internal region, or the 3' end of RNA molecules. Claim 32 is drawn to the nucleic acid being genomic DNA. Claim 33 is drawn to the population being normalized. Claim 34 is drawn to the first subset of

nucleic acids being separated by size. Claims 35, 36, and 37 are drawn to the separation being done by polyacrylamide gel electrophoresis or agarose gel electrophoresis. Claims 38-42 are drawn to the members of the first subset differing by a given number. Claim 47 is drawn to the method of claim 27, wherein the library is prepared by a process comprising: a) ligating the isolated first subset of nucleic acid molecules to a vector to form a population of vector-insert nucleic acid molecules; b) transforming the vector-insert nucleic acid molecules into a host cell to form a library; and c) culturing the library under conditions that allow for at least some members of the library to be distinguished from other members of the library. Claims 48 and 49 are drawn to the members being spatially distinguishable. Claim 50 is drawn to members being combined prior to separating the second subset. Claim 51 is drawn to the second subset being separated by size. Claim 52 is drawn to them being separated by electrophoresis. Claim 53 is drawn to electrophoresis being in a replaceable matrix formulation comprising a) a linear polyacrylamide (LPA) solution, wherein the LPA concentration in said solution ranges between 1% to 3% (w/w); b) at least one denaturant; c) a buffer; and d) 3 M to 8 M urea, wherein the formulation is capable of separating nucleic acids. Claim 54 is drawn to the nucleic acids being compared to one or more known nucleic acids prior to sequencing. Claim 55 is drawn to the nucleic acids being pooled prior to sequencing.

Kamb et al. teaches "Gene libraries, usually cDNA or genomic, can be constructed in a variety of vectors including plasmid and viral vectors by methods well-established in the art... The library vectors can be designed to propagate on one or

more of a variety of cell types including bacteria, yeast, or mammalian cells. In some cases the libraries are intended to be as representative of the nucleic acids present in a particular organism or tissue as possible. These are termed total genomic or cDNA libraries. In other cases the libraries are intended to contain only a subset of sequences; for example, those sequences that are prevalent in one cell type and absent in another. Such limited libraries can be constructed using, for example, cDNA from one source that has been treated with subtraction or blocking procedures as suggested above to remove sequences held in common with a second source. See, supra. Libraries have traditionally been used in two ways; for biochemical screens and for genetic screens. The process of screening allows isolation of sequences of interest from the bulk of library sequences. Biochemical screens require a probe, either a nucleic acid probe or a protein probe such as an antibody (in the case of expression libraries). Specific genes or gene fragments can be fished out of a library using an appropriate probe. Genetic screens permit recovery of sequences from a library of genes or gene fragments which complement or rescue a particular mutant phenotype using an appropriate selection scheme. For example, if a yeast genomic library is introduced into HIS3-yeast cells and plated on media lacking histidine, only cells that have acquired library sequences that contain a functional HIS3 gene will be able to grow. These growing colonies can be treated such that the resident library sequences are recovered. A number of ways can be envisioned to enrich and identify differentially expressed library members. For example, Representational Difference Analysis (RDA) permits the purification of sequences that differ substantially between two samples because, e.g., they

contain a restriction fragment length polymorphism..." (Cols. 26 and 27). Therefore, Kamb teaches providing a population of nucleic acid molecules comprising at least a first subset of nucleic acid molecules, which comprises a second subset of nucleic acid molecules (this is inherent, since the second subset was not defined, it would encompass any part of the first subset, including a single nucleotide), separating and isolating the first subset, constructing a library containing the second subset, recovering nucleic acids from the subset, and isolating a nucleic acid molecule from the second subset (this could be accomplished by simply excising any part of the first subset, or amplifying a part of the first subset).

Kamb does not teach sequencing the fragment after recovery, or the use of gel electrophoresis to separate the fragments based on size, or normalizing the population.

Okayama et al. teaches using gel electrophoresis to separate the various sizes, and DNA sequencing of the sequences after being in the library. (Page 164, second Col.)

It would have been obvious to one of ordinary skill in the art at the time of the invention to sequence the fragments after recovery, or to run them in a gel and discriminate between them based on size. This is because these additional methods would have provided the actual sequence information, which would have been very helpful in determining the origin and usefulness of that individual sequence. Furthermore, normalizing would have been obvious because it would have allowed for underrepresented members of the mRNA population to be sequenced.

9. Claims 43-46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kamb et al. in view of Okayama et al. as applied to claim 27 above, and further in view of Macevicz et al. (USPN 6,136,537).

The claims, which depend from claim 27, are drawn to the population of nucleic acids comprising nucleic acids having terminal sequences identical to those produced by digestion of a nucleic acid molecule with one or more restriction enzymes. Claim 44 is drawn to that restriction endonuclease being a Type II or a Type IIS restriction endonuclease. Claims 45 and 46 are drawn to the endonuclease recognizing a four or six nucleotide recognition site.

As disclosed above, Kamb et al. teaches "Gene libraries, usually cDNA or genomic, can be constructed in a variety of vectors including plasmid and viral vectors by methods well-established in the art... The library vectors can be designed to propagate on one or more of a variety of cell types including bacteria, yeast, or mammalian cells. In some cases the libraries are intended to be as representative of the nucleic acids present in a particular organism or tissue as possible. These are termed total genomic or cDNA libraries. In other cases the libraries are intended to contain only a subset of sequences; for example, those sequences that are prevalent in one cell type and absent in another. Such limited libraries can be constructed using, for example, cDNA from one source that has been treated with subtraction or blocking procedures as suggested above to remove sequences held in common with a second source. See, supra. Libraries have traditionally been used in two ways; for biochemical screens and for genetic screens. The process of screening allows isolation of sequences of interest

from the bulk of library sequences. Biochemical screens require a probe, either a nucleic acid probe or a protein probe such as an antibody (in the case of expression libraries). Specific genes or gene fragments can be fished out of a library using an appropriate probe. Genetic screens permit recovery of sequences from a library of genes or gene fragments which complement or rescue a particular mutant phenotype using an appropriate selection scheme. For example, if a yeast genomic library is introduced into HIS3-yeast cells and plated on media lacking histidine, only cells that have acquired library sequences that contain a functional HIS3 gene will be able to grow. These growing colonies can be treated such that the resident library sequences are recovered. A number of ways can be envisioned to enrich and identify differentially expressed library members. For example, Representational Difference Analysis (RDA) permits the purification of sequences that differ substantially between two samples because, e.g., they contain a restriction fragment length polymorphism..." Therefore, Kamb teaches providing a population of nucleic acid molecules comprising at least a first subset of nucleic acid molecules, which comprises a second subset of nucleic acid molecules (this is inherent, since the second subset was not defined, it would encompass any part of the first subset, including a single nucleotide), separating and isolating the first subset, constructing a library containing the second subset, recovering nucleic acids from the subset, and isolating a nucleic acid molecule from the second subset (this could be accomplished by simply excising any part of the first subset, or amplifying a part of the first subset).

Kamb does not teach sequencing the fragment after recovery, or the use of gel electrophoresis to separate the fragments based on size.

Okayama et al. teaches using gel electrophoresis to separate the various sizes, and DNA sequencing of the sequences after being in the library.

Neither Kamb nor Okayama et al. teaches the use of a restriction endonuclease that is a Type II or Type IIS.

Macevicz et al. teach that "Preferably, the method of the invention comprises the steps of i) providing a population of polynucleotides having predetermined ends; ii) inserting each polynucleotide of the population into a vector, so that the vector has at least one type IIs restriction endonuclease recognition site adjacent to each end of the inserted polynucleotide, each type IIs restriction endonuclease recognition site being oriented such that a type IIs restriction endonuclease recognizing either site cleaves the vector interior to the inserted polynucleotide; iii) cleaving each vector with one or more type IIs restriction endonucleases recognizing the type IIs restriction endonuclease recognition sites so that the vector is linearized and has a sequence tag of the inserted polynucleotide at each end; iv) re-circularizing the vector to form a pair of sequence tags for the inserted polynucleotide; and v) determining the nucleotide sequence of each pair of sequence tags of a sample of re-circularized vectors. Preferably, the population of polynucleotides having predetermined ends is produced by digesting a cDNA library with one or more frequent-cutting restriction endonucleases, e.g. restriction endonucleases each having a four-base recognition sequences. Preferably, the pairs of sequence tags are tabulated to form a frequency distribution of sequences

in the population of polynucleotides which may be used directly, or related to the frequency distribution of sequences in another population, such as a cDNA library, from which the analyzed population is derived. In one aspect of the invention, the pairs of sequence tags are excised from the re-circularized vectors and ligated together to form a concatemers, which are cloned in a conventional sequencing vector. " (Col. 2, lines 32-65).

It would have been obvious to one of ordinary skill in the art at the time of the invention to use the method of Macevicz et al. to excise certain portions of the nucleic acid from the vector library. This is because it would have allowed for specific sequences to be isolated, purified, and sequenced, if desired.

Summary

Claim 44 is objected to. Claims 27-55 are rejected under 35 U.S.C. 112, second paragraph. Claims 27-55 are rejected under 35 U.S.C. 103(a). No claims are free of the prior art.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Janell Taylor Cleveland, whose telephone number is (703) 305-0273.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached at (703) 308-1152.

Any inquiries of a general nature relating to this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted by facsimile transmission. Papers should be faxed to Group 1634 via the PTO Fax Center using (703) 305-3014 or 305-4227. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG (November 15, 1989.)

Janell Taylor Cleveland

April 11, 2001

W. Gary Jones
Supervisory Patent Examiner
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4/13/01